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13. ABSTRACT (Maximum 200 words)

This report describes exploratory development of the fiber optic metal ion biosensor initially developed under ONR contract N00014-91-J-1572. The anticipated application is to monitor wastewater outflows to verify the success of treatment procedures for removing Cu(II); of special interest are wastewater outflows from drydocks resulting from hull blasting and washing operations, which contain ppm levels of Cu and Zn prior to treatment. Issues in this phase of development include the response speed and reversibility of the sensor, transducer stability, and the presence of interferents in treated wastewater. We found that the sensor responded in less than one minute to part per trillion levels of free Cu(II), that it was reversible at the expected rate, that treated wastewater could be measured, and that the apoprotein was storable for months.

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FINAL REPORT

Grant No. N00014-98-J-0685

PRINCIPAL INVESTIGATOR: Richard B. Thompson

INSTITUTION: University of Maryland School of Medicine

GRANT TITLE: Exploratory Development of Metal Ion Biosensor

AWARD PERIOD: 8 May 1998 to 7 May 2000

OBJECTIVE: The goal of this program is to develop a fiber optic metal ion biosensor for cations such as Cu(II), Zn(II), and others dissolved in wastewater treated and released by the Navy. For applications of Navy interest, it is desired that the sensor exhibit high sensitivity and selectivity, as well as a rapid, reversible response in real time. An application of particular interest was the monitoring of the cleanup procedure used to remove copper contamination from dry dock/graving dock wastewater arising from antifouling paint removed during washdown and hull blasting operations. While the current cleanup procedure is satisfactory, it is important to monitor the effluent to assure that no contaminated water comes through the system due to leakage or exhaustion of the ion exchange cartridges.

The thrust of this phase of the program was to begin addressing issues of importance in development of the sensor for these practical applications. Among these issues were the speed and reversibility of the sensor, operability in sea water (as a demanding model for wastewater), identification of potential interferents by testing in treated wastewater, and stability of the transducer.

APPROACH: Details of the approach are given in the Final Report to grant No. N00014-91-J-1572, "Fiber Optic Metal Ion Biosensor," and references therein. Briefly, the sensor transducer reversibly binds the metal ion of interest and transduces the binding as a change in fluorescence intensity, lifetime, or anisotropy. This change in fluorescence reflects the fractional occupancy of the binding site by the metal, which is controlled by and thus reflects its concentration. The transducer is a biologically-derived molecule, a variant of the human enzyme carbonic anhydrase II, which has unmatched affinity and selectivity for the metal ions of interest. The transducer molecule is covalently attached to the distal end of the optical fiber (which is in contact with the wastewater stream), and its fluorescence is interrogated and collected through the optical

fiber by the instrument at the proximal end. For Cu(II) especially, transduction as a change in anisotropy or lifetime is preferred because these are freer from artifact and much easier to calibrate; lifetimes can also be easily measured through optical fiber, which thus permits remote sensing.

ACCOMPLISHMENTS: In large measure, we successfully addressed the issues of concern in this portion of the program. The sensor responds rapidly (under a minute) and reversibly to picomolar levels of free copper (II) ion in sea water and artificial sea water models. The sensor is reversible, but the off rate (which is as fast as possible given the sensor's affinity) is significantly slower, responding in tens of minutes (as expected). We found that copper levels in treated wastewater from a Navy ship maintenance facility (Puget Sound Naval Shipyard) were approximately 0.25 nM, indicating that the cleanup process under development at NFESC met the specification, and that there were no significant interferents present in the treated wastewater. Our stability tests indicated that the apoprotein was stable in solution under refrigeration for some months.

CONCLUSIONS: We conclude that a fluorescence-based fiber optic metal ion biosensor is feasible for real time determination of free copper in wastewater streams at very low levels. A key goal in the next phase of the program ("Metal Ion Biosensor for Wastewater Discharge," N00014-00-1-0921) is demonstration of response to a rapidly changing picomolar Cu(II) concentration in sea water.

SIGNIFICANCE: The significance of our results for this and the related work is that we demonstrate that a biosensing approach can measure trace amounts of free copper (II) in treated wastewater which is salty. Measuring these low levels in such a complex matrix in real time demonstrates the feasibility of monitoring a wastewater outflow to assure that copper levels are maintained within the required range, or to cue maintenance and avoid release of treated wastewater not meeting specifications. Moreover, as hull washing robots are developed (see the work of G. Bohlander at NSWCCD), it becomes reasonable to treat the Cu-contaminated wastewater they generate on-line, since a greater fraction of copper can be removed by filtration if the water is filtered promptly, thereby minimizing the soluble copper which must be removed by other means.

PATENT INFORMATION: See report on contract N00014-91-J-1572 for issued patents.

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS:

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